Example 5: Encapsulation of a Dendrimer-Susceptible Test Compound Complex

[0182] In one example, a sequestering agent like alginate was used to encapsulate or embed a dendrimer-ethanolamine complex. Dendrimers are hyper-branched synthetic macromolecules that can be made using controlled sequential processes to give them defined structural and molecular weight characteristics. They are reported to be useful not only to protect sensitive substances, but mainly for improving on timed release of embedded components within a given medium. Further, when cell recognition or cell attachment moieties are incorporated into dendrimers, the dendrimer may specifically attach to a cell and deliver its contents. This is highly useful in targeted delivery of a valuable substance, or an extremely labile substance, or highly sensitive cell regulatory molecules. This study was done to see if microencapsulation of a dendrimer-labile molecule complex stabilizes the molecule from degradation, and further, whether the labile substance can be stored for long periods of time under ambient conditions like room temperature while associated with a dendrimer in the dry state. The procedure for forming dendrimers was done as described in Astruc et al., Chem. Rev. 2010, 110:1857-1959, which is hereby incorporated by reference in its entirety. Briefly, different types of dendrimers can be synthesized based on the core structure that initiates the polymerization process. The microencapsulated dendrimers of this disclosure are described in FIG. **6**. In one embodiment, the dendrimer was encapsulated in a capsule of alginate. Alginate protects the dendrimers and upon reconstitution, releases the dendrimers with the sensitive substance. Alginate is retained on the membrane filter during filtration. The exposed dendrimer can target the sensitive substance to a cell due to the presence of cell recognition sites present on the dendrimer, thereby improving availability of sensitive or low-concentration substances such as growth factors like insulin, vitamins, etc. to the cell. The results are shown in FIGS. **6**, **7***a*, **7***b*, **8**, **9** and **10**.

[0183] In FIG. **8**, the unprotected ethanolamine potency dropped quickly in AGT™ under ambient conditions while maintaining potency for many months longer when protected by microencapsulation (microbeads). Microencapsulated ethanolamine continued to show no change in potency even up to ten months and beyond at ambient temperatures (see FIG. **8**).

[0184] Further examples for microencapsulating labile compounds other than ethanolamine are shown below. A sequestering agent like alginate was used to encapsulate or embed a dendrimer-insulin complex. The labile compound insulin was polymerized with PAMAM dendrimer, and further microencapsulated in alginate microbeads with or without PLL. Briefly, for dendrimer generation, PAMAM 2.5 (carboxylate surface groups) was used. Insulin was conjugated onto the dendrimer to which RGD sequences were also added to to target the cell surface. The ratios of components in this complex was dendrimer:RGD:insulin=1:7:12.

[0185] Protocol for Dendrimer Conjugation:

[0186] Concentration of conjugate and alginate was adjusted as on attachment. Next conjugate was added to the alginate and mixed several minutes to distribute evenly. It was dripped through a 26 guage needle using a lml syringe into a calcium chloride bath (13.32 g/L). It was left to incubate for 30 minutes to solidify microcapsules. Then rinsed 1× in a solution also containing the dendrimer conjugate to prevent loss of component from the microcapsules. Then dried overnight in a vacuum over dessicant. The loaded microbeads were then added to AGT dry-format medium or used by adding directly into a liquid medium.

[0187] Insulin-Dendrimer-RGD Dilution Protocol

[0188] The insulin concentration of the dendrimer-insulin-RGD conjugate was estimated to be ~2 mg/ml (pg 165/168). It needed to be diluted in half to get to ~1 mg/ml (1000 μg/ml).

[0189] Stock r-insulin for assays was set at 1000 μg/ml, which is a 100× of a 10 μg/ml concentration. This was equivalent to a 1000× of a 1 μg/ml concentration, which was well within the titerable insulin concentration with the 96-well assay (pg. 160). So the effective dilution of the insulin used for microencapsulation (a, pg 168) is 1:1000×. In addition, 1:1000 was what we set for the microencapsulation equivalency (1 ml of alginate beads was used for 1000 ml of medium). The Eugene conjugate control would also be diluted first 1:1 (above) and then used at 1:1000 dilution.

[0190] We needed to add the dendrimer-insulin-RGD conjugate (liquid) to 3% alginate so as to yield 2% alginate final. (1+0.5). This dilution did not impact the dendrimer-insulin-RGD conjugate amount since all the dendrimer added would go into the beads and ultimately into the specified medium. Example: 0.1 ml of dendrimer-insulin-RGD conjugate was used for 100 ml of final medium volume (at 1:1000). Added this 0.1 ml dendrimer-insulin-RGD to 0.2 ml of 3% alginate to equal 2% alginate. Used all of it by dripping into calcium bath and processing as on pg. 167 and adding to 100 ml medium. Similarly, the positive insulin control (b, pg. 168) was diluted to 1:1000× not to 10 μg/ml.

[0191] As shown in FIG. **7**A, microencapsulation of the dendrimer showed substantially enhanced stability at room temperature compared to the unencapsulated dendrimer with insulin, or with insulin alone (no dendrimer, no encapsulation with alginate). The potency of insulin in the dendrimer was comparable to that of insulin alone at 4° C. In addition, cell growth studies on the mammalian HeLa cell line with insulin alone (control), the dendrimer containing—microbeads (test), or microbeads without dendrimer all showed equivalent potency and growth characteristics of HeLa cells, and both the dendrimer or the alginate did not interfere with viability or growth of Hela cells, showing that the microencapsulation procedure was not toxic for cells (FIG. **7**B). Accelerated shelf life studies showed that microencapsulation (microbead) improved insulin stability in dry format media for up to one week at 37° C. (FIG. **10**).

[0192] In a further example, vitamins (for e.g., thiamine, vitamin B12, etc.) were used as test labile compounds for microencapsulation and addition to dry format media (FIG. **9**). A sequestering agent like alginate was used to encapsulate or embed a dendrimer-vitamin complex, as described above for encapsulated insulin. As seen from FIG. **9**, accelerated shelf life studies showed that microencapsulation greatly improved vitamin stability, for two weeks at 37° C.

[0193] Microencapsulation can extend storage conditions of sensitive compounds like ethanolamine, vitamins, growth factors, etc., which can positively impact shipping and handling of dry media formulations like AGT™, etc., at room temperature rather than at lower temperatures (for e.g., refrigeration or dry ice), which can decrease shipping costs. Microencapsulation of labile compounds in dry format